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13. ABSTRACT ***(Maximum 200 Words)***

Since returning from the Gulf War (GW), veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen. This phenomenon, referred to as "Burning Semen Syndrome" (BSS), is similar to symptoms experienced by civilian women diagnosed with localized seminal plasma hypersensitivity (SPH). Desensitization of women with localized SPH using their sexual partner's seminal plasma proteins (SPP) has been successful suggesting an IgE-mediated mechanism.[1-4] The objectives of this project were to identify the prevalence of BSS, to clinically evaluate GW couples with BSS, to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature and to identify potential treatment(s) for BSS. Among civilians with localized SPH, the male is typically asymptomatic and the female's symptoms are alleviated with a condom. Couples with BSS differ in that the male often has burning after contact with his semen and less than half of the females get relief with a condom. Furthermore, many GW couples exhibit features of GW syndrome which confounds their evaluation of BSS symptoms. Civilian and GW couples produce heterogeneous antibody responses. However, unlike civilian couples, GW couples have not improved symptomatically after SPP desensitization. Thus far, although BSS parallels the clinical symptoms manifested by civilians with localized SPH, these two disorders appear to be distinctly different.

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Table of Contents:

I. Introduction:

Since returning from the Gulf War (GW), veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen. This phenomenon referred to as "Burning Semen Syndrome" (BSS) is similar to symptoms experienced by civilian women diagnosed with localized seminal plasma hypersensitivity. These women experience localized vaginal inflammation, characterized by burning and pain which occurs immediately after contact with their sexual partner's semen. Desensitization using relevant homologous seminal plasma protein antigens obtained from their sexual partner has been successful in many cases suggesting that some post-coital localized vaginal reactions may be IgE-mediated.[1-3] A questionnaire survey previously distributed to 1,073 women suspecting they might have symptoms consistent with localized and/or systemic seminal plasma protein hypersensitivity revealed that 12% fulfilled the diagnostic criteria for this disorder.[4] This survey indicated that seminal plasma protein hypersensitivity reactions were more common than previously reported.[4] The objectives of this research project were to identify the prevalence of BSS, to evaluate GW veterans and their sexual partners with BSS, to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature and to identify potential treatment(s) for BSS.

II. Body:

A. Experimental Methods/Procedures

Questionnaires:

A web page was established on the Internet to identify GW veterans deployed to the Persian Gulf with and without BSS which includes two screening questionnaires to be completed by the GW veteran and their sexual partner, respectively. All individuals who responded to the screening questionnaires were sent more detailed questionnaires to further elucidate details about their symptoms and GW exposures. A separate detailed questionnaire was sent to the male and female. This questionnaire packet also included screening surveys for post-traumatic stress disorder (PTSD). Our program coordinator made frequent follow-up phone calls to encourage the completion and return of all questionnaires promptly.

Clinical Evaluation of GW veterans:

Gulf War veterans and their sexual partners who consent to participate in this project were required to undergo screening blood tests and cultures to exclude bacterial, fungal and viral infections or other medical disorders (ie. diabetes mellitus, chronic yeast infections, prostatitis...) which could be causing or contributing to their symptoms. All GW veterans and their sexual partners were skin tested using the "prick" method to assess their allergic status. Skin testing was performed to box elder (tree), fescue (grass), short ragweed, *Alternaria* (outdoor mold), *Mucor* (indoor mold), cat, and dust mite in addition to a positive histamine and negative saline control. A fresh ejaculate was collected from each male at the time of their initial evaluation. A small portion of the ejaculate was used for prick skin testing of the male and female in order to

determine if either elicited an immediate hypersensitivity reaction. The remaining portion of the sample was sent for semen cultures. All females were asked to undergo a pelvic examination which included a pap smear, vaginal and/or cervical cultures. Finally, serum and an additional semen specimen is obtained from the male and serum from the female to screen for specific IgG and IgE antibodies to the male's seminal plasma proteins and to other unrelated male seminal plasma proteins by ELISA.

Processing of Semen:

Semen specimen were allowed to liquify at room temperature for 1 hr, and the pH is checked. The specimen is transferred to a high-speed centrifuge tube and an equal volume of phosphate-buffered saline (for specimens that are to be used for treatment) or Tris-buffered saline (for specimens to be used for analytical purposes) was added. The specimen was centrifuged at 30,000 X G for 1 hr at 4°C in a JA-14 rotor in a Beckman J2-21M high speed centrifuge. The supernatant fluid, whole seminal plasma, (which is usually either a pale straw color or completely clear) was removed, leaving approximately 1 ml of fluid to avoid removing any pelleted material. The pellet was suspended in 1 ml of PBS or Tris buffered saline and allowed to soak in the fluid overnight at 4°C. The next day the pellet was vortexed to liquify the material and immediately frozen at -75°C. In those cases where the seminal plasma was to be used for future treatment, the sample was dialyzed against PBS with three exchanges of the outer dialysis fluid. The seminal plasma was then aliquoted and frozen at -75°C.[2] Previous comparison studies evaluating SDS-PAGE protein patterns of fresh whole seminal plasma to pooled ejaculates collected and stored over several days revealed no differences in their protein patterns.

Direct Competitive ELISA:

Specific IgG and IgE ELISA was performed using whole seminal plasma obtained from the GW male subject and asymptomatic civilian male controls. A Costar flat-bottom, 96-well polystyrene plate (Corning) was coated with 100 µl of seminal plasma protein previously diluted to concentration of 10 µg/ml with 0.15 mol/L NaCl. The plate was incubated for two hours at room temperature with 0.15 mol/L tween-phosphate buffer saline to block for unreacted sites. Both the GW veteran and their sexual partner's serum was diluted 1:5 and added in triplicate to the microtiter wells. The plate was allowed to incubate for 24 hours at room temperature. For IgG antibody detection, alkaline phosphatase conjugated goat anti-human IgG (Sigma) were diluted to 1:2000 and added to each well. After the plate incubated for one hour at room temperature, 100 µl of 1 mg/ml p-nitrophenyl phosphate substrate was added to each well. The enzyme reaction was allowed to proceed for 30 minutes and then stopped with KOH. The optical density of each well was measured using a microplate ELISA reader at 405 nm. For IgE antibody detection, goat anti-human IgE (Kirkegard and Perry) diluted to 1:1000 was added to each well and incubated for one hour at room temperature. The plate was then washed and alkaline-phosphatase labeled rabbit anti-goat IgG diluted to 1:2000 was added to each well. After the plate incubated for one hour at room temperature, the optical density was determined as described for IgG isotype specific antibody.

Column Chromatography of Seminal Plasma:

Ten ml of seminal plasma was chromatographed on a Sephacryl S-200 HR [High Resolution] Hi-Prep 26/60 column (Amersham Pharmacia Biotech) using PBS, pH 7.4, as the running buffer. The column was controlled with a computerized FPLC unit, and the absorbance of the effluent was monitored at 280 nm. Fractions of 5 ml were collected and fraction pools were made of the peaks according to the readout in the UV chromatogram. Separate columns were used for civilians and Persian Gulf War veterans, but each column was cleaned with 0.25M NaOH-1M NaCl between patient specimens. Molecular weights were estimated by comparison with a set of known molecular weight standards (also from Pharmacia), which were run monthly when the columns were in use.

Gel Electrophoresis:

Gel electrophoresis was performed on a Pharmacia Phast Electrophoresis unit, using 6/4 or 8/1 gel combs. Staining of the gels was also performed on the Phast Unit using the staining module. Most gels were silver stained to take advantage of the high sensitivity of this type of stain. With the small volume and relatively low concentrations of proteins used in this study, Coomassie blue or Amido black staining does not possess the required sensitivity.

Immunoblotting:

Whole seminal plasma, electrophoresed on a 12.5% acrylamide gel, was transferred to polyvinylene difluoride (PVDF) membranes using the Pharmacia Phast system. This membrane allows better retention of low molecular weight proteins <50kd. Immunoblots were blocked using non-fat dry milk at 37° C for 2.5 hours, followed by the addition of either the male or female sera for incubated for one hour at room temperature. After washing with tris buffered saline containing 0.5% Tween-20, anti-human IgG alkaline phosphatase conjugate was added and incubation was allowed for 1 hour at room temperature. After washing NBT/BCIP substrate was added and incubated at room temperature for 30 minutes. The membranes were washed using distilled water and air dried at room temperature.

Polymerase Chain Reaction (PCR) and Southern Blotting for Ureaplasma urealyticum DNA:

PCR was performed on DNA isolated from the seminal pellet for the presence of DNA of *Ureaplasma urealyticum.* The DNA was extracted using a procedure for extraction of DNA from sperm provided by the Qiagen Corporation, using their QIAamp Tissue Kit (Cat. No. 29304). The sequences for the 20-mer PCR primers for the urease gene of *U. urealyticum* (termed UU1 and UU2) and PCR methods were adapted from Krieger, et al. (J. Clin. Microbiol. 34:3120-3128, 1996) and prepared by a commercial supplier. Control DNA from two strains of *U. urealyticum,* 9R and 27817, was supplied by Dr. George Kenny (University of Washington).

Amplified DNA was separated on a 2% Nu-Sieve agarose gel and stained with ethidium bromide for visualization. The DNA was blotted through to a nylon membrane (Magnagraph) using a neutral Southern blot procedure in a S&S TurboBlotter downward transfer apparatus. The DNA was detected with a 20-mer probe 3'-tailed with biotin-dCMP and developed using the Life Technologies Photogene™ assay kit for chemiluminescent detection of the biotin probe.

Cell Proliferation Assays:

Cell proliferation assays were performed on peripherial blood mononuclear cells isolated from blood of both partners. Cells are islolated in Accuspin® tubes using Histopaque®-1077 (both from Sigma Diagnostics). The isolated PBMC's are quantitated by the Clinical Hematology Laboratory at University Hospital. 1 X 10^6 cells are placed in the wells of a 96-well cell culture plate (Costar), in 100 µl of complete medium (RPMI-1640 contain in 10% fetal bovine serum). 100 µl of whole seminal plasma at dilutions of 1:10 and 1:100 are added to the cells, and controls of no additive (medium alone) and phytohemaglutinin (PHA) at 10 µg/ml are also added. The plate is sealed and incubated at 37°C for 5 days. The proliferation of the cells is quantitated using the 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit III from Boehringer Mannheim (Catalog No. 1444611).

Affinity Chromatography

The globulin fraction of serum from GW and spouses is precipitated with 40% saturated ammonium sulfate, using two separate precipitations. The globulins are dissolved in PBS, pH 7.4 and held at -20°C until used. Immediately prior to affinity chromatography the globulins are dissolved in coupling buffer (0.2 M $NaHCO_3$-0.5 M NaCl, pH 8.3) by running through a PD-10 column (Amersham Pharmacia Biotech), which contains Sephadex G-25, equilibrated in the buffer. The concentration of globulins is adjusted to 10 mg/ml, as determined using the Pierce BCA Protein Assay. 1 ml of globulin (10 mg) is coupled to the matrix of a Hi-Trap NHS [N-hydroxysuccinamide]-activated column, 1 ml size at room temperature for 30 min, and the excess material is washed out and unbound coupling sites blocked with successive washes with 0.5 M ethanolamine-0.5 M NaCl alternating with 0.1 M Na acetate-0.5 M NaCl. The coupling efficiency of this method is 95%.

Whole seminal plasma (WSP) is transferred to adsorption buffer (0.075 M Tris-HCl, pH 8.0) in a PD-10 column, and run through the coupled column at a flow rate of 0.2 ml per minute. Those proteins of the WSP which are not bound to specific antibody in the coupled globulins are collected in a separate tube, and the column is rinsed with 15 ml of adsorption buffer. The specifically adsorbed protein(s) are then eluted with 25 ml of elution buffer (0.1 M glycine-HCl, 0.5 M NaCl, pH 2.7), and 5 ml fractions are collected. The fractions are concentrated on an Amicon minicon-CS15 concentrator (15,000 MW cut-off).

Statistical Methods

Statistical analyses presented in Tables 4-6 were based on respondents' distinct "yes" or "no" answers. Missing or otherwise unknown responses were excluded from analysis. An alpha level of .05 was used for all statistical tests. Unless otherwise specified, a Student's t-test, chi-square or Fisher Exact test was used when appropriate and there was a subsequent failure to reject the null hypothesis in each case.

B. Results

Identification of BSS Subjects and Cohort Control Groups:

The first aim of this project was to identify GW veterans with BSS. This required establishing contacts with GW screening physicians at local and remote Veterans Administration Hospitals, veteran's organizations such as the American Legion, AmVets, and Veterans of Foreign Wars and

other advocates of GW veterans. A significant amount of time was devoted to publicizing this project to the news media in order to inform the general public and GW veteran population about BSS. Several magazines (ie. Men's Health, Science News, Playboy...) and newspapers published reports on BSS. Major radio and television news wires (i.e. Reuters, NBC) aired stories regarding BSS. This media exposure successfully heightened the public's awareness of BSS and our investigation of this problem in GW veterans. However, the most effective means of identifying this population has been through an internet web site. A control population of healthy asymptomatic GW veterans has been more difficult to identify due to a lack of cooperation or disinterest in this project. Unless they were directly affected with BSS most GW veterans were too embarrassed to participate in this study even with the opportunity to earn a stipend.

A cohort civilian population of women (n=36) with symptoms that meet the criteria for localized and/or systemic seminal plasma hypersensitivity have subsequently been identified. We have also identified normal civilian men and women which have been used as controls in measuring specific IgG and IgE antibodies to seminal plasma proteins. This group is comprised of a mixture of vasectomized and non-vasectomized males. Previously, we have not found that vasectomies have an influence on symptoms experienced by GW couples with BSS or civilian couples diagnosed with seminal plasma protein hypersensitivity reactions.

Prevalence of BSS and Seminal Plasma Protein Hypersensitivity:

The Cincinnati VAH has been selected as one of 11 centers participating in a multicenter project designed to randomly evaluate the health of1,000 GW families. When completed, approximately 11,000 couples will have completed questionnaire surveys. As a co-investigator of this project, specific questions about BSS were included in this survey. The information collected from these questionnaire responses should provide a fairly accurate prevalence of BSS among GW couples.

In order to determine an accurate prevalence of seminal plasma protein hypersensitivity among civilian women, a screening questionnaire about seminal plasma hypersensitivity was distributed to local gynecologist/obstetrician offices. We anticipated over 1,000 responses to this questionnaire from women in the Greater Cincinnati area. However, questionnaire responses were very poor which is a reflection of either the low prevalence of this problem in the general population or an uneasiness by patients and or physicians with the subject matter contained in the questionnaire.

Questionnaire Responses:

Table 1 summarizes compliance among GW and civilian couples completing questionnaires. To date, 195 GW veterans have responded to one or more questionnaires about BSS. In general, compliance was poor with completing the more extensive questionnaires that elicited detailed information about their condition and general health (n=46 which is less than 25%). This is in sharp contrast to civilian couples who were 100% compliant in completing all of the questionnaires they were sent.

Table 2 provides demographic information obtained from 183 GW couples who completed the initial screening questionnaire. This population was comprised of GW veterans from 39 states, Puerto Rico, Canada and the United Kingdom. Over 20% of GW veterans were anonymous in their responses to this questionnaire. Several reasons for their anonymity exist. Many respondents were still active military and were concerned about jeopardizing their careers in the military and many were

very embarrassed about the nature of their problem and personal questions they were asked. The majority of GW male veterans (69%) experienced burning after contact with their own semen and an even greater percent of their sexual partners were symptomatic (87%). Only 8% of these couples experienced symptoms prior to going to the GW and 50% developed symptoms with first sexual contact after returning from the GW. Only 42% experienced relief of symptoms with use of a condom which is in contrast to what has been observed for civilians with seminal plasma protein hypersensitivity. Surprisingly, only 42% of GW couples had sought medical attention for their symptoms at the time they completed the questionnaire.

Table 3 summarizes the responses of GW couples to more detailed questionnaires designed to obtain information about exposures while in the GW and their general state of health. Their responses to post-traumatic stress disorder questionnaires are also summarized. The average age of GW males was 35 y/o and the average age of their sexual partner was 33 y/o. Only 26% of GW males compared to 61% females reported that their general health was good or better. Ninety-four percent reported some type of exposure while in the GW. The greatest exposure was to ingestion of pyridostigmine bromide of which 35% reported side effects to this medication. Among the population of GW couples that completed the more detailed questionnaires, only 26% had onset of their symptoms immediately after returning from the GW compared to 50% of the larger group of respondents (n=183). Symptoms began within minutes after seminal fluid contact $\geq$90% for both males and females. Symptoms were transient for males but persisted for days in 33% of female respondents. Symptoms were abated with the use of condoms in only 44% of male and 36% of female respondents suggesting that BSS is quite different from seminal plasma protein hypersensitivity. However, many GW couples were strongly averse to using condoms. When condoms were used, they were often only placed prior to ejaculation and not during actual intercourse. Therefore, the female was not completely protected from seminal fluid contact because of leakage that may have occurred during intercourse. Both GW males and their sexual partners reported histories of allergies within line of the estimated prevalence reported in the general population. Interestingly, almost 50% of females reported a history of frequent vulvovaginal candidiasis.

Several interesting observations were made when the population of GW male respondents were broken down into healthy verses unhealthy groups. An unhealthy status was defined as having multiple somatic symptoms suggestive of GW syndrome whereas a healthy status was defined no other physical complaints other than BSS symptoms. Greater than 2/3rds of this population were classified as being unhealthy. GW males with isolated BSS had statistically significant lower reporting of pesticide exposure or involvement in decontamination operations. They also reported significantly less likely to experience symptoms after direct exposure to their own semen compared to their unhealthy counterparts. Furthermore, the unhealthy group of GW males were significantly more likely to be undergoing treatment for PTSD compared to their healthy counterparts.

Table 5 compares characteristics of GW female sexual partners with BSS symptoms to a cohort population of civilian women experiencing either localized or systemic seminal plasma protein hypersensitivity reactions. Civilian women were significantly more likely to have a personal and family history of atopy compared to the female sexual partners of GW veterans. Furthermore, only 34% of GW female sexual partners met the criteria for diagnosis of seminal plasma protein hypersensitivity which requires complete relief of symptoms with use of a condom. There was no difference between the two groups with respect to age, type or duration of symptoms experienced,

number of sexual partners or predisposing conditions previously associated with seminal plasma protein hypersensitivity reactions. When these two groups were further broken down into localized responders verses systemic responders (see Table 6) there was no statistically significant difference between those civilian and GW female sexual partners reporting systemic symptoms. Interestingly, civilians reporting localized symptoms had a greater likelihood for having a personal or family history of atopy compared to their GW counterparts. Civilians were also more likely to fulfill the criteria for a diagnosis of localized seminal plasma protein hypersensitivity which requires prevention of symptoms with use of a condom.

Exposure Assessment of BSS GW Veterans:

Identification of one or more toxic exposures responsible for or associated with BSS symptoms has been difficult to establish. As in all retrospective studies, it has been very difficult to determine if actual past chemical and/or biologic exposures occurred and if so whether they were related to BSS. A geographical information system (GIS) data base developed by Dr. Jack Heller, a senior scientist in charge of the deployment environmental exposure surveillance program at the U.S. Army Center for Health Promotion and Preventive Medicine, has been generated which allows estimations of GW veteran exposures while they were in the Persian Gulf using both modeled and sampled data. GW veterans were tracked from the time they entered the GW arena up until the time they left the region. This model has been criticized because it required making several assumptions about the veteran's exposure. Furthermore, sampling was not initiated until several months after the GW began. However, this GIS represents the best model to identify GW veteran outliers that might be at more risk for developing exposure related health problems. Figures 1 compares the modeled and sampled cancer risk levels of a group of GW veterans with BSS (n=19) to the risk levels of all deployed troops during the GW. Both modeled and sampled cancer risk levels for the BSS population were at or below the maximum estimated risk for all GW deployed troops. In this model, cancer risks for all GW deployed troops and the BSS GW veteran subpopulation were below the acceptable cancer risk levels established by the Environmental Protection Agency. Figure 2 compares the modeled and sampled noncancer risk for GW veterans with BSS and all GW deployed troops. Noncancer risk levels pertain primarily to all health related problems other than cancer as a result of oil fire particulate exposure. The GW BSS subpopulation noncancer risk was at or below the estimated noncancer risk for all GW deployed groups. Table 7 lists all of the air pollutants that GW veterans had potential exposure with during their tour.

Laboratory Screening of GW Veterans and Their Sexual Partners:

Less than 50% of GW couples with BSS had sought medical attention for their symptoms prior to their enrollment in this study. Many of the GW male veterans had a cursory GW evaluation at a regional Veterans hospital or at a military hospital. However, these evaluations were very non-specific and did not ask questions about BSS symptoms. Therefore, none of the GW males or their female sexual partners had adequate laboratory testing performed to exclude obvious underlying causes of their symptoms such as sexually transmitted diseases. As most of our subjects lived out of state, their evaluation of BSS depended on cooperation from their local or regional Veterans or military hospitals. This required identifying a physician who would assist us in obtaining serum from both the male and female in addition to semen from the male and vaginal samples from the female in order to complete all of the necessary screening tests to exclude underlying etiologies as outlined in

our original proposal. When it was possible GW couples were invited to Cincinnati to complete their evaluation. This process proved to be very time consuming and difficult to accomplish for several reasons. First, the GW couples expressed a great deal of distrust for their local Veterans hospital (VAH) because of how they had been previously treated and evaluated and secondly, there were no provisions within the VAHs that paid for the necessary screening tests for the GW male or their female sexual partner. After significant amount of lobbying, we were able to identify ways in which the male and female could have this testing completed which would be covered by the VAH. Those that refused to have the testing at the VAH were instructed to have it performed by an outside physician which was paid for by this grant. However, special allocations had to be made to cover these costs since the original budget did not include allocations for screening laboratory testing as it was assumed that many of these tests would have been previously performed to exclude an underlying cause.

Table 8 summarizes abnormal laboratory test results obtained for a subgroup of GW couples where one or both members had experienced BSS symptoms. Several of the female sexual partners of GW veterans had significant ANA titers and positive cervical cultures for yeast, streptococcus or ureaplasma urealyticum (a strain of Mycoplasma). One women had a significantly elevated sedimentation rate which seem to correlate with an active mycoplasma infection. Treatment with two weeks of doxycycline of three GW couples where the female had positive ureaplasma cervical cultures did not effect their BSS symptoms. This lack of response may reflect the length of treatment with antibiotics and not its effectiveness. Completed laboratory screening results of GW couples are still slowly being received in our laboratory. A larger population of GW couples with BSS requires treatment with antibiotics to determine if these symptoms are related to an infectious etiology.

Column Chromatography:

Whole seminal plasma from GW males and civilian males fractionated by column chromatography thus far are illustrated in figures 3 and 4, respectively. Figure 3 includes the spectrographic patterns of 10 GW veterans. These patterns were all very similar. Figure 4 illustrates the spectrographic patterns of civilian males whole seminal plasma which, in general, exhibited very similar peak distribution to what was observed for the BSS GW veterans. Two civilian males elicited very small peaks that correlated to lower molecular weight proteins compared to other civilian males and the BSS GW veterans.

ELISA for specific IgG and IgE antibodies to seminal plasma proteins:

Tables 9 and 10 summarize specific IgG and IgE ELISA results for nine GW couples. The shaded areas represent specific antibody responses by the GW male and their sexual partner (if they had one) to the GW male's whole seminal plasma proteins. In general, there was a heterogeneous response among the GW couples tested. In some cases neither the male or female produced antibody responses, in some cases only the female or male elicited antibodies and in some cases both the female and males elicited antibody responses. IgG responses were more pronounced than IgE responses. Gulf war couple #3 exhibited antibody responses that most closely resembled what is encountered for civilian couples with seminal plasma protein hypersensitivity. These antibody responses are similar to those responses observed among civilian couples with probable seminal plasma protein hypersensitivity (Figure 5).

Figure 6 illustrates specific IgG and IgE antibody responses, respectively, to seminal plasma

responses for a GW male and his sexual partner. The male was asymptomatic and the female exhibited isolated localized vaginal burning after contact with semen. The female produced a significant amount of IgG and IgE antibodies to her spouse's seminal plasma proteins. Interestingly, antibody responses were also observed to negative control pooled serum (negative serum refers to serum obtained from asymptomatic non-GW controls). Figure 7 represents specific IgG and IgE responses by a civilian male and female to the male's seminal plasma protein fractions. The female also presented with isolated localized vaginal burning after contact with semen. Neither the male or female produced specific antibody to the male's seminal plasma proteins. A similar antibody response was observed for the negative pooled serum against the civilian male's seminal plasma protein fractions.

SDS-PAGE and Western blotting:

Figure 8 illustrates the SDS-PAGE of whole seminal plasma obtained from GW and civilian men. In general, a very similar protein pattern has been observed for all subjects. Figure 9 represents an SDS-PAGE gel of a GW veteran's whole seminal plasma before and after fractionation and figure 10 represents a western blot for specific IgG antibody of this gel. Specific IgG was found for proteins with molecular weights of 45kd, 50 kd, 80kd and 180kd. Figure 11 represents an SDS-PAGE gel of whole seminal plasma and fractionated proteins from a civilian male whose sexual partner had systemic seminal plasma protein hypersensitivity. Figure 12 illustrates the western blot for IgG antibody of this gel. Specific IgG antibodies were identified to several proteins (8-12 proteins) ranging from molecular weights of <10kd to 200kd. SDS-PAGE gels prepared using the whole seminal plasma and fractionated proteins obtained from several GW veteran and civilian males exhibit very similar patterns (Figures 9 and 11). However, specific IgG and IgE immunoblots exhibit a heterogeneous pattern indicating that antibodies are being produced to several proteins.

PCR and Southern blotting:

We previously used a PCR technique to detect the presence of *Ureaplasma urealyticum* in the semen of GW veterans. Preliminary PCR results of probing DNA isolated from the semen of GW veterans and civilian controls with a specific Ureaplasma urealyticum urease primer was unsuccessful. We therefore sent DNA samples of GW and control civilian couples to an outside laboratory to determine if mycoplasma was related to BSS. Thus far none of these samples have contained mycoplasma, however, the results are still pending for many couples. It is important to note that two women who had positive cervical cultures for mycoplasma infection that were treated with Doxycycline for one month had no improvement in their symptoms.

Cell Proliferation:

Cell proliferation experiments using whole seminal plasma from the GW male and fresh peripheral blood mononuclear cells from a normal donor are currently in progress. Significant proliferation was not observed in three subjects studied thus far. Additional cell proliferation experiments are being performed using seminal plasma protein fractions to determine if high molecular weight protein fractions suppress lymphocytic responses normally as previously described and to determine whether low molecular weight proteins, to which specific antibody responses are commonly found, induce proliferation. The objective of these experiments is to investigate the role of lymphocytes in regulating specific antibody responses associated with BSS.[3,4]

Discussion:

The results thus far illustrate distinct similarities as well distinct differences between GW and civilian couples who exhibit burning after contact with semen. There differences are outlined below. The cause of BSS remains unknown. Over the next six months we will focus on four points: 1) complete evaluation (including complete questionnaires, blood work, specific antibody responses) of a larger group of GW couples; 2) cell proliferation assays to determine any differences between GW and civilian couples with SPP hypersensitivity; 3) affinity chromotography (or 2-D electrophoresis followed by mass spectroscopy) to identify specific proteins eliciting antibody responses and finally; 4) treatment of a subpopulation of GW couples with desensitization to seminal plasma proteins when appropriate to confirm or exclude the relationship of antibody responses with BSS.

III. Key Research Accomplishments:

- Less than 50% of BSS couples have relief with use of a condom
- Personal and Family history of atopy is significantly lower among BSS couples compared to civilians
- Lower incidence of PTSD among GW couples with isolated BSS compared to GW couples with multiple symptoms suggestive of GW syndrome
- A higher incidence of reported exposure to pesticides and decontamination procedures among GW couples with multiple symptoms compared to GW couples with isolated BSS
- Exposure levels leading to cancer and non-cancer health risks among GW couples with BSS is no greater than that reported for all United States deployed troops to the GW based on a geographical information system model
- Many female sexual partners of GW males have asymptomatic vaginal infections
- Seminal plasma proteins obtained from GW males with BSS are structurally the same as seminal plasma proteins obtained from civilian male controls
- Heterogeneous antibody responses are observed to whole seminal plasma proteins and fractionated seminal plasma proteins for GW and civilian couples
- Antibody responses seem to correlate better to disease pathogenesis in civilian couples with seminal plasma protein hypersensitivity as they respond better symptomatically to SPP desensitization compared to GW couples with BSS

IV. Reportable Outcomes:

Abstracts (Appendix A) were presented at the Society of Toxicology meeting held in Cincinnati, March 1997, the American Academy of Allergy, Asthma and Immunology (AAAAI) in Washington D.C. in 3/98 and at the AAAAI for 3/99.[5-7] Abstracts were submitted in 1998 and 1999 for the Gulf War investigator meeting held in Washington, D.C.

V. Conclusions:

The hypothesis of this project was to determine whether Burning Semen Syndrome was a disorder similar to localized seminal plasma protein hypersensitivity. Both of these disorders have similar clinical presentations which consists of localized vaginal burning and pain immediately after contact with semen. Among civilians with localized SPP hypersensitivity, the male is typically asymptomatic and the female's symptoms go away with use of a condom.[1,8] Couples with BSS often have quite different clinical presentations in that the male often complains of burning after contact with their own semen and less than half of the females have relief of their symptoms with a condom. Furthermore, many of the GW males and their female sexual partners exhibit features of GW syndrome which confounds their evaluation of BSS symptoms to an even greater extent. In general, both civilian and GW couples produce heterogeneous antibody responses.[7] However, civilian couples have responded to seminal plasma protein desensitization compared to GW couples who thus far have not improved symptomatically.[1] Whether antibody responses are involved in the pathogenesis of BSS or are an epiphenomenon remains to be determined. Cell proliferation assays should shed light on whether GW couples with BSS elicit abnormal lymphocytic responses compared to civilian couples. Investigation into a chronic infectious etiology of BSS is also still in progress.

Thus far, it appears that BSS is a heterogeneous disorder. In some instances it parallels the symptoms manifested by civilians with localized SPP hypersensitivity but in other cases it is a very different disorder. This project has been extended to allow further evaluation, characterization and treatment of a larger group of GW couples. Final conclusions will be reported at that time.

VI. References:

1) Bernstein JA, Herd Z, Bernstein DI, Korbee L, Bernstein IL. Evaluation and Treatment of Localized Vaginal Immunoglobulin E-Mediated Hypersensitivity to Human Seminal Plasma. Obstet Gynecol 1993;82:667-73.

2) Bernstein IL, Englander BE, Gallagher JS, Nathan P, Marcus ZH. Localized and Systemic Hypersensitivity Reactions to Human Seminal Plasma Fluid. Annals of Int Med 1981;94:459-465.

3) Friedman SA, Bernstein IL, Enrione M, Marcus ZH. Successful Long-Term Immunotherapy for Human Seminal Plasma Anaphylaxis. JAMA 1984;251:2684-87.

4) Bernstein JA, Sugumaran R, Bernstein DI, Bernstein IL. Prevalence of Human Seminal Plasma Hypersensitivity Among Symptomatic Women. Ann Allergy Asthma Immunol 1997; 78:54-8.

5) Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model For Gulf War "Burning Semen Syndrome". Fundamental and Applied Toxicology 1997;37:201.

6) Bernstein JA. Evaluation of Persian Gulf War Veterans and Their Sexual Partners with Burning Semen Syndrome. J Allergy Asthma and Clin Immunol 1998; 101:S80.

7)Bernstein JA, Perez,AS, Frazier KM, Floyd R. Antibody Responses in Clinical Couples with Seminal Plasma Protein Hypersensitivity and Gulf War Couples with Burning Semen Syndrome. J Allergy Asthma and Clin Immunol 1999; 103:S226.

8) Presti ME, Druce HM. Hypersensitivity Reactions to Human Seminal Plasma. Annals of Allergy 1989; 63:477-482.

Table 1. Summary of compliance among Gulf War couples and civilian couples completing questionnaires.

Gulf War Veterans and Partners	
Total number of study participants - GW Veterans	195
-- Participants not interested in further participation	64
-- Participants excluded (lost to study, HIV, etc.)	2
Completing Screening Questionnaire #1	183
Completing Questionnaire #2 - Females	60
Completing Questionnaire #2 - Males	31
Completing Extended Questionnaire #3 - Females	39
Completing Extended Questionnaire #3 - Males	46
Completing PTSD Surveys	
-- Combat Exposure Scale	44
-- Mississippi PTSD Rating Scale	37
Civilian Seminal Plasma Hypersensitivity Patients	
Total number of participants - Positive Control Group	36
Number ofParticipants post treatment	7
Completing Questionnaire #2 - Females	36

Table 2. Summary of screening questionnaire positive responses from GW veterans.[1]

Total Number of Respondents	183
Anonymous Respondents	39
Responses from Web Site	137
Geographic Distribution	39 States, Puerto Rico, Canada, UK
Reaction to Semen Contact (including ejaculation)	69%
Sexual Partner has Reaction to Semen	87%
Symptoms Pre-existed Gulf War Experience	8%
Symptoms Began Immediately After Return from GW	50%
Condoms Eliminate Reactions	44%
Previously Sought Medical Attention	42%
Treated for Sexually Transmitted Diseases Since GW	13%

1 Unanswered responses assumed to be "no".

Table3. Summary of gulf war couple responses to extended questionnaire & PTSD surveys.

Responses	Male = 46	Female = 39
Average age	35	33
Average length of tour	5.3 months	------
Location while in Persian Gulf	Iraq, Kuwait, Saudi Arabia	------
Reported chemical exposures	94 %	------
Average length of exposure	Varied	------
Diagnosis of Leishmaniasis	4 %	------
Treatment for Leishmaniasis	0 %	------
Uranium exposure	37 %	------
Exposure to biological agents	61 %	------
Ingestion of Pyridostigmine Bromide	70 %	------
Side effects from Pyridostigmine Bromide	35 %	------
Exposure to pesticides	52 %	------
Received vaccinations	59 %	------
Previous Evaluation for Post-traumatic Stress Disorder[1]	46 %	------
Previous Treatment for Post-traumatic Stress Disorder[1]	26 %	------
Respondents negative for PTSD [2]	46 %	------
Respondents possible for PTSD [2]	24 %	------
Repondents probable for PTSD [2]	30 %	------
Involvement in decontamination operations	30 %	------
Current state of health	26 % good or better	61 % good or better
Sexually transmitted disease	15 %	8 %
Reaction to semen	63 %	97 %
Sexual partner has reaction	89 %	------
Onset of reaction with first sexual encounter after returning from GW	26 %	39 %
Time onset of symptoms occur	"Minutes" for 92% of men effected	"Minutes" for 90 %
Length of time symptoms persist	"Minutes" for 46 % of men effected	"Minutes" for 21 % "Days" for 33 %
Systemic symptoms	52 %	64 %
Condoms eliminate reactions	44 %	36 %
History of vasectomy	22 %	------
History of infertility problems	9 %	------
History of Allergies	26 %	33 %
Food Allergies	13 %	13 %
Drug Allergies	20 %	36 %
Same sexual partner pre/post GW	52 %	74 %
Recurrent vaginal yeast infections	------	49 %
Current use of oral contraceptives	------	15 %

1 Based on self reported history.
2 Based on Mississippi PTSD Rating Scale (N=37).

Table 4. Summary of healthy and unhealthy gulf war veterans' responses to extended questionnaire & PTSD surveys.

Responses	Healthy = 14	UnHealthy = 31	ρ
Average age	31	36	=.017
Average length of tour	4.6 months	5.1	
Location while in Persian Gulf	Iraq, Kuwait, Saudi Arabia, PG Sea	Iraq, Kuwait, Saudi Arabia,	
Reported chemical exposures	100%	90 %	
Average length of exposure	Varied	Varied	
Diagnosis of Leishmaniasis	0 %	7 %	
Treatment for Leishmaniasis	0 %	0 %	
Uranium exposure	43 %	36 %	
Exposure to biological agents	50 %	65 %	
Ingestion of Pyridostigmine Bromide	79 %	65 %	
Side effects from Pyridostigmine Bromide	36 %	39 %	
Exposure to pesticides	29 %	62 %	=.006
Received vaccinations	57 %	62 %	
Previous Evaluation for PTSD	7 %	65 %	=.001
Previous Treatment for PTSD[1]	0 %	39 %	=.003
Respondents negative for PTSD [2]	57 %	26 %	
Respondents possible for PTSD [2]	29 %	16 %	
Repondents probable for PTSD [2]	7 %	36 %	
Involvement in decontamination operations	7 %	42 %	=.019
Current state of health	86 % good or better	0 % good or better	<.001
Sexually transmitted disease	7 %	19 %	
Reaction to semen	29 %	68 %	=.034
Sexual partner has reaction	100 %	84 %	
Sexual partner has systemic reaction to semen	29 %	32 %	
Onset of reaction with first sexual encounter after returning from GW	21 %	29 %	
Time onset of symptoms occur	"Minutes" for 50% of men effected	"Minutes" for 91% of men effected	
Length of time symptoms persist	"Minutes" for 50 % of men effected	"Minutes" for 43 % of men effected	
Systemic symptoms	50 %	57 %	
Condoms eliminate reactions	43 %	42 %	
History of vasectomy	14 %	26 %	
History of infertility problems	7 %	10 %	
History of Allergies	7 %	36 %	
Food Allergies	0 %	19 %	
Drug Allergies	14 %	23 %	
Same sexual partner pre/post GW	43 %	58 %	

1 Based on self reported history.
2. Based on Mississippi PTSD Rating Scale (N=37).

Table 5. Comparison of GW Females with BSS to Civilian Females with SPH ♦

ITEM	Partners of GW Veterans N=59	Civ. Women N=36	ρ
Age of Onset:			
<30	.68	.72	
>31	.32	.22	
Unknown	0	.06	
Reactions:			
Urticaria/Pruritus	.68	.64	
Chest Tightness/Dyspnea/ Cough/Wheezing	.29	.44	
Dizziness/Faintness	.34	.36	
Complete Collapse/Unconscious	.03	.14	
Local Pain/Burning	.90	.81	
Redness/Rash/Blisters	.95	.72	
Onset of Symptoms:			
0-60 minutes	.86	.94	
>60 minutes	.14	.06	
Unknown	0	0	
Duration of Symptoms:			
<24 hours	.51	.47	
>24 hours	.48	.53	
Unknown	.02	0	
Prevented by Condom:			
Yes	.36	.75	
No	.24	.17	
Unknown	.41	.08	
Atopy:			
Yes	.39	.67	ρ=.007
No	.61	.28	
Unknown	0	.06	
Multiple Partners:			
Yes	.12	.22	
No	.86	.78	
Unknown	.02	0	
Predisposing Conditions:			
First Intercourse	.37	.36	
History of :			
Pregnancy, Gyn/Urological Surgery	.27	.28	
Unknown	.56	.33	
Family History of Atopy:			
Yes	.34	.67	ρ=.003
No	.64	.31	
Unknown	.02	.03	
Diagnosis of SPH:			
Probable	.34	.72	ρ=.002
Possible	.22	.17	
Undetermined	.44	.14	

♦ Chi -square or Fisher Exact analysis excluding "Unknown" responses

Table 6. Comparison by subtype of GW Females with BSS to Civilian Females with SPH ♦

ITEM	GW Partners Systemic Sxs N=25	Civ. Women Sytemic Sxs N=19	GW Partners Localized Sxs N=34	Civ. Women Localized Sxs N=17
Age of Onset:				
<30	.76	.68	.62	.77
>31	.24	.32	.38	.12
Unknown	0	0	0	.12
Reactions:				
Urticaria/Pruritus	.88	.84	.53*	.41*
Chest Tightness/Dyspnea/ Cough/Wheezing	.68	.84	–	–
Dizziness/Faintness	.80	.68	–	–
Complete Collapse/Unconscious	.08	.26	–	–
Local Pain/Burning	.84	.63	.94	1.00
Redness/Rash/Blisters	.80	.58	.77	.88
Onset of Symptoms:				
0-60 minutes	.84	.95	.88	.94
>60 minutes	.16	.05	.12	.06
Unknown	0	0	0	0
Duration of Symptoms:				
<24 hours	.40	.26	.59	.71
>24 hours	.56	.74	.41	.29
Unknown	.04	0	0	0
Prevented by Condom:				
Yes	.36	.74	.35	.77
No	.32	.16	.18	.18
Unknown	.32	.11	.47	.06
Atopy:				
Yes	.52	.74	.29	.59
No	.48	.26	.71	.29
Unknown	0	0	0	.12
Multiple Partners:				
Yes	.16	.32	.09	.12
No	.80	.68	.91	.88
Unknown	.04	0	0	0
Predisposing Conditions:				
First Intercourse	.44	.26	.32	.47
History of :				
Pregnancy, Gyn/Urological Surgery	.32	.32	.24	.24
Unknown	.52	.26	.59	.41
Family History of Atopy:				
Yes	.40	.63	.29	.71
No	.56	.32	.71	.29
Unknown	.04	.05	0	0
Diagnosis of SPH:				
Probable	.36	.68	.32	.71
Possible	.32	.16	.15	.18
Undetermined	.32	.16	.53	.12

♦ Chi -square or Fisher Exact analysis excluding "Unknown" responses □ $p < .05$

* Women identifying only pruritus as a systemic symptom were classified as localized responders.

Table 7. Complete listing of pollutant names and acronyms [3]

Pollutant Abbreviation	Pollutant Name	Pollutant Abbreviation	Pollutant Name
Acne [2]	Acenaphthene	Hg [2]	Mercury
Acny [2]	Acenaphthylene	Hptn [2]	Heptane
Al	Aluminum	Ipyr [2]	Ideno(1,2,3-cd)pyrene
Anth [2]	Anthracene	Mg [2]	Magnesium
As [2]	Arsenic	MNaph1 [2]	1-methylnapthalene
Bant [2]	Benzo(a)anthracene	MNaph2 [2]	2-methylnapthalene
Bapy [2]	Benzo(a)pyrene	Mpxy	Meta-para Xylene
Bbfl [2]	Benzo(b)fluoranthene	Mxyl [2]	Meta-xylene [1]
Be [2]	Beryllium	Na [2]	Sodium
Benz [2]	Benzene [1]	Naph [2]	Naphthalene [1]
Bepy [2]	Benzo(e)pyrene	Ni [2]	Nickel [1]
Bghi [2]	Benzo(g,h,i)perylene	NO [2]	Nitrogen Oxide
Bkfl [2]	Benzo(k)fluoranthene	NO2 [2]	Nitrogen Dioxide
Bphnl [2]	Biphenyl	NO3 [2]	Nitrates
Ca [2]	Calcium	O3 [2]	Ozone
Cd [2]	Cadmium	Oxyl [2]	Ortho-xylenes [1]
Chry [2]	Chrysene	Pb [2]	Lead
Cl	Chlorine	Phen [2]	Phenanthrene
Chl [2]	Chlorides	PM10	Particulate Matter <10um
Cr3 [2]	Chromium(3)	Prpb [1 2]	Propylbenzene
Cr6 [2]	Chromium(6)	Pxyl [2]	Para-xylene [1]
Crblz [2]	Carbazole	Pyre [2]	Pyrene
Dban [2]	Dibenzo(ah)anthracene	SO2 [2]	Sulfur Dioxide [1]
Dbfrn [2]	Dibenzofuran	SO4 [2]	Sulfates
Dmnpt [2]	2,6-dimethylnapthalene	Tolu [2]	Toluene [1]
Ethlb [2]	Ethylbenzene [1]	TSP	Total Suspended Particulate [1]
Fe [2]	Iron [1]	V [2]	Vanadium [1]
Flan [2]	Fluoranthene	Zn [2]	Zinc
Fluo [2]	Fluorene		

[1] Modeled pollutants of concern
[2] Sampled pollutants of concern
[3] Additional sampled pollutants of concern: Acid Gasses (Acetic, Formic, Hydrochloric, Nitric, Sulfuric)

Table 8. Summary of Pertinent Positive Laboratory Results of GW Veterans and Their Sexual Partners Evaluated

Subject	Laboratory Test Result	Male (GW Veteran)	Female
1 (-) PTSD	ANA Serum Mycoplasma IgG Ab Serum HSV-1 IgG Ab Serum CMV IgG Ab Cervical Urea.urealyticum	Positive (1:40) Positive	Positive 1:160 speckled Positive Positive Positive Positive
2 poss. PTSD	ANA Serum Mycoplasma IgG Ab Serum HSV-1 IgG Ab Serum CMV IgG Cervical Urea. urealyticum Urine Group B strep.	 Positive Positive Positive	Positive 1:80 Positive Positive Positive Positive Positive (10-50,000 cfu/ml)
3 (-) PTSD	Serum Mycoplasma IgG Ab Serum CMV IgG Ab Cervical pap smear	Positive Positive	 Positive Positive for Candida yeast
4 (-) PTSD	WSR Bands on differential Serum HSV-1 IgG Cervical Urea. urealyticum		68 mm/hr (nl=0-20) 14% (nl=0-6) Positive Positive
5 poss. PTSD	Serum HSV-1 IgG Ab Serum HSV-2 IgG Ab Cervical culture Cervical pap smear	Positive Positive	Positive Moderate Strep Group B Many inflammatory cells
6 (+) PTSD	Serum HSV-1 IgG Ab	Positive	Not available (wife did not participate in evaluation)
7 (+) PTSD	Cervical Cytologic Material		Acute Inflammation
8 (-) PTSD	Serum CMV IgG	Positive	Positive

Table 9. IgG to Whole Seminal Plasma in Gulf War Couples

Whole Seminal Plasma Protein

Serum	GW1	GW2	GW3	GW4	GW5	GW6	GW7	GW8	GW9
Sxatic Ctrl	0.62	0.77	0.26	1	0.23	0.31	0.15	0.15	0.4
Asxatic Ctrls	0.06	0.22	0.16	0.63	0.11	0.09	0.08	0.11	0.43
GW1M	0.88	0.37	1.08	0.05	0.8	0.93	0.8	0.89	0.32
GW1F	0.4	0.14	0.95	0.03	0.29	0.26	0.28	0.21	0.43
GW2M	0.08	0.03	0.42	0	0.07	0.05	0.03	0.02	0.1
GW2F									
GW3M	0.91	1.03	0.27	0.37	0.14	0.15	0.11	0.16	0.29
GW3F	0.74	0.51	1.06	0.2	0.68	0.94	1.07	1.02	0.48
GW4M	0.54	0.36	0.39	0.06	0.25	0.19	0.19	0.2	0.18
GW4F	0.07	0.08	0.09	0.86	0.08	0.06	0.08	0.11	0.22
GW5M	0.52	0.53	0.45	0.2	0.12	0.14	0.12	0.13	0.27
GW5F	0.04	0.06	0.07	1.1	0.04	0.04	0.02	0.02	0.31
GW6M	0.25	0.1	0.22	0	0.12	0.19	0.11	0.15	0.28
GW6F	0.17	0.08	0.18	0.03	0.2	0.13	0.07	0.07	0.18
GW7M	1.7	1.92	0.9	0.93	0.11	0.18	0.1	0.14	0.25
GW7F	0.4	0.51	0.23	0.57	0.02	0.16	0.12	0.38	0.28
GW8M	1	1.17	0.12	0.06	0.08	0.05	0.03	0.05	0.34
GW8F	0.25	0.26	0.24	0.99	0.16	0.17	0.15	0.18	0.3
GW9M	0.46	0.53	0.26	0.58	0.24	0.05	0.1	0.06	0.49
GW9F	0.16	0.35	0.05	0.92	0.03	0.02	0.01	0.02	0.28

Shaded areas represent specific IgG responses of the GW male and their sexual partner to the male's whole seminal plasma protein.

Sxatic Ctrl = serum from a symptomatic GW female with consistent IgG responses to WSP
Asxatic Ctrls = pooled sera of laboratory workers

Table 10. IgE to Whole Seminal Plasma in Gulf War Couples

Whole Seminal Plasma Protein

Serum	GW1	GW2	GW3	GW4	GW5	GW6	GW7	GW8	GW9
Sxatic Ctrl	0.79	0.98	0.21	1	0.22	0.09	0.14	0.14	0.4
Asxatic Ctrls	0.45	0.67	0.15	0.14	0.17	0.1	0.08	0.3	0.4
GW1M	0.35	0.21	0.59	0.06	0.63	0.03	0.47	0.54	0.25
GW1F	0.01	0.1	0.26	0.04	0.23	0.04	0.17	0.13	0.38
GW2M	0	0.03	0.35	0.01	0.06	0.005	0.14	0.28	0.09
GW2F									
GW3M	0.8	1.33	0.1	0.11	0.11	0.01	0.08	0.15	0.21
GW3F	0.34	0.35	0.62	0.13	0.61	0.6	0.78	0.78	0.37
GW4M	0.02	0.21	0.18	0.06	0.08	0.06	0.06	0.11	0.11
GW4F	0	0.04	0.22	0.04	0.11	0.04	0.06	0.07	0.23
GW5M	0.02	0.24	0.06	0.07	0.09	0.02	0.03	0.06	0.2
GW5F	0	0.07	0.06	0.2	0.08	0.01	0.09	0.08	0.21
GW6M	0	0.09	0.18	0.03	0.18	0.12	0.49	0.09	0.29
GW6F	0	0.13	0.2	0.09	0.17	0.12	0.08	0.1	0.18
GW7M	1.7	1.65	0.22	0.41	0.24	0.06	0.06	0.08	0.26
GW7F	0	0.22	0.06	0.14	0.13	0.12	0.07	0.21	0.21
GW8M	0.47	0.8	0.17	0.03	0.21	0.12	0.04	0.11	0.41
GW8F	0	0.15	0.16	0.28	0.16	0.07	0.1	0.16	0.19
GW9M	1.16	1.55	0.41	0.17	0.13	0.09	0.18	0.13	0.36
GW9F	0.13	0.29	0.11	0.62	0.12	0.16	0.06	0.06	0.45

Shaded areas represent specific IgE responses of the GW male and their sexual partner to the male's whole seminal plasma protein.

Sxatic Ctrl = serum from a symptomatic GW female with consistent IgE responses to WSP
Asxatic Ctrls = pooled sera of laboratory workers

Figure 1. Comparison of Troop Unit & Burning Semen Syndrome (BSS) Study Cancer Risk Levels to Acceptable EPA Risk Levels

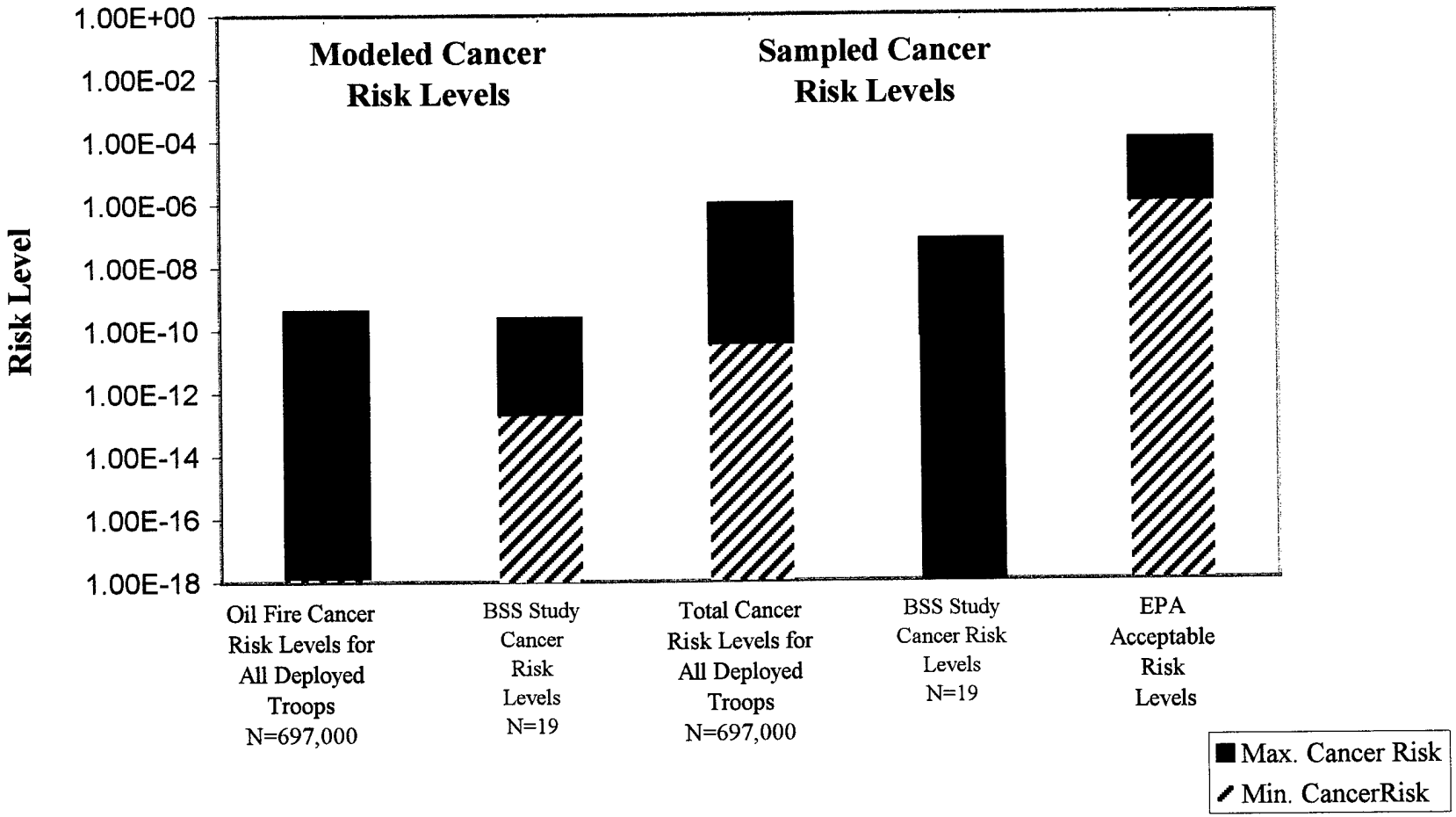


Figure 2. Comparison of Troop Unit & Burning Semen Syndrome (BSS) Study Non-Cancer Risk Levels to Acceptable EPA Risk Level

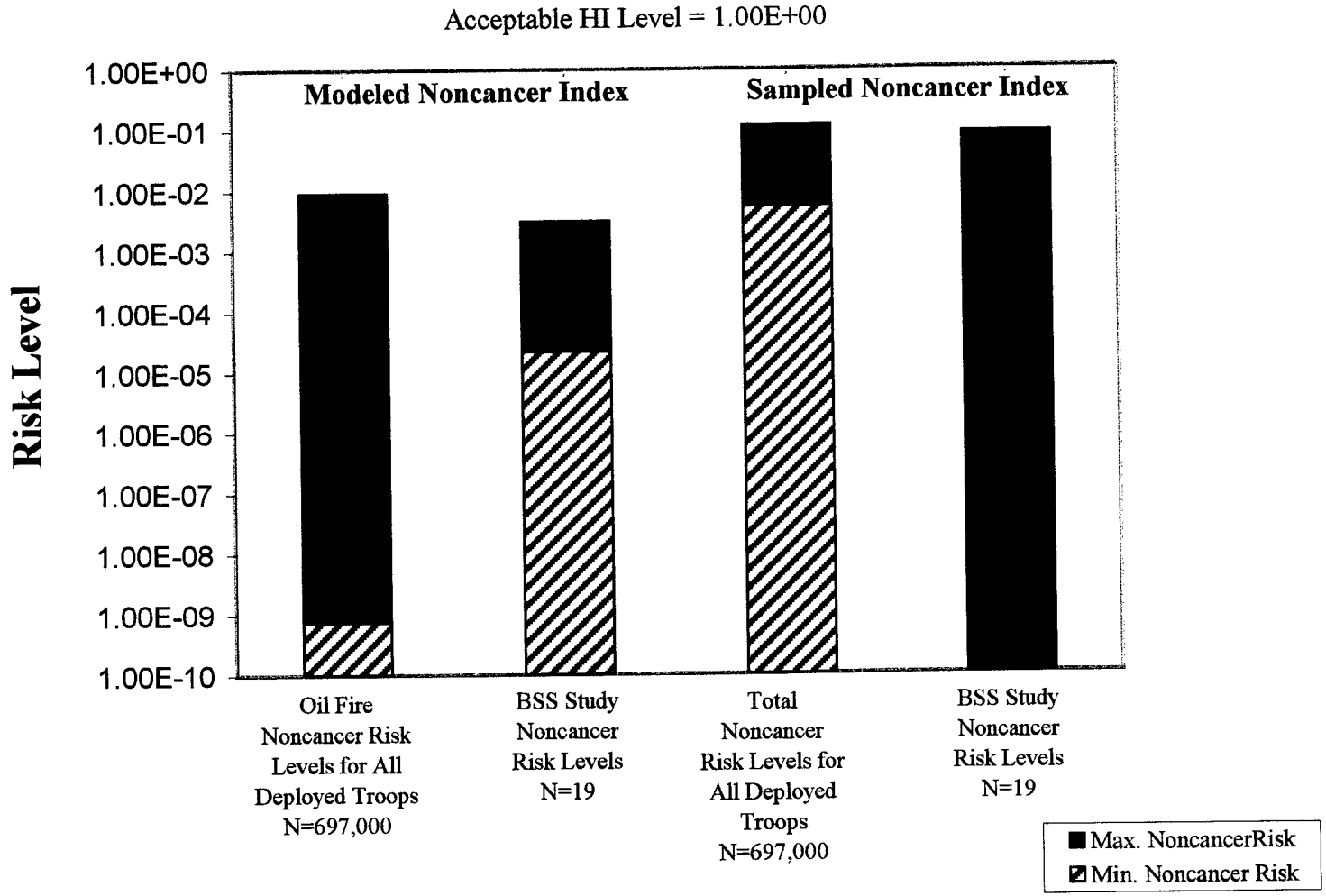


Figure 3. Chromatography of Whole Seminal Plasma from a sample of GW Veterans with Burning Semen Syndrome

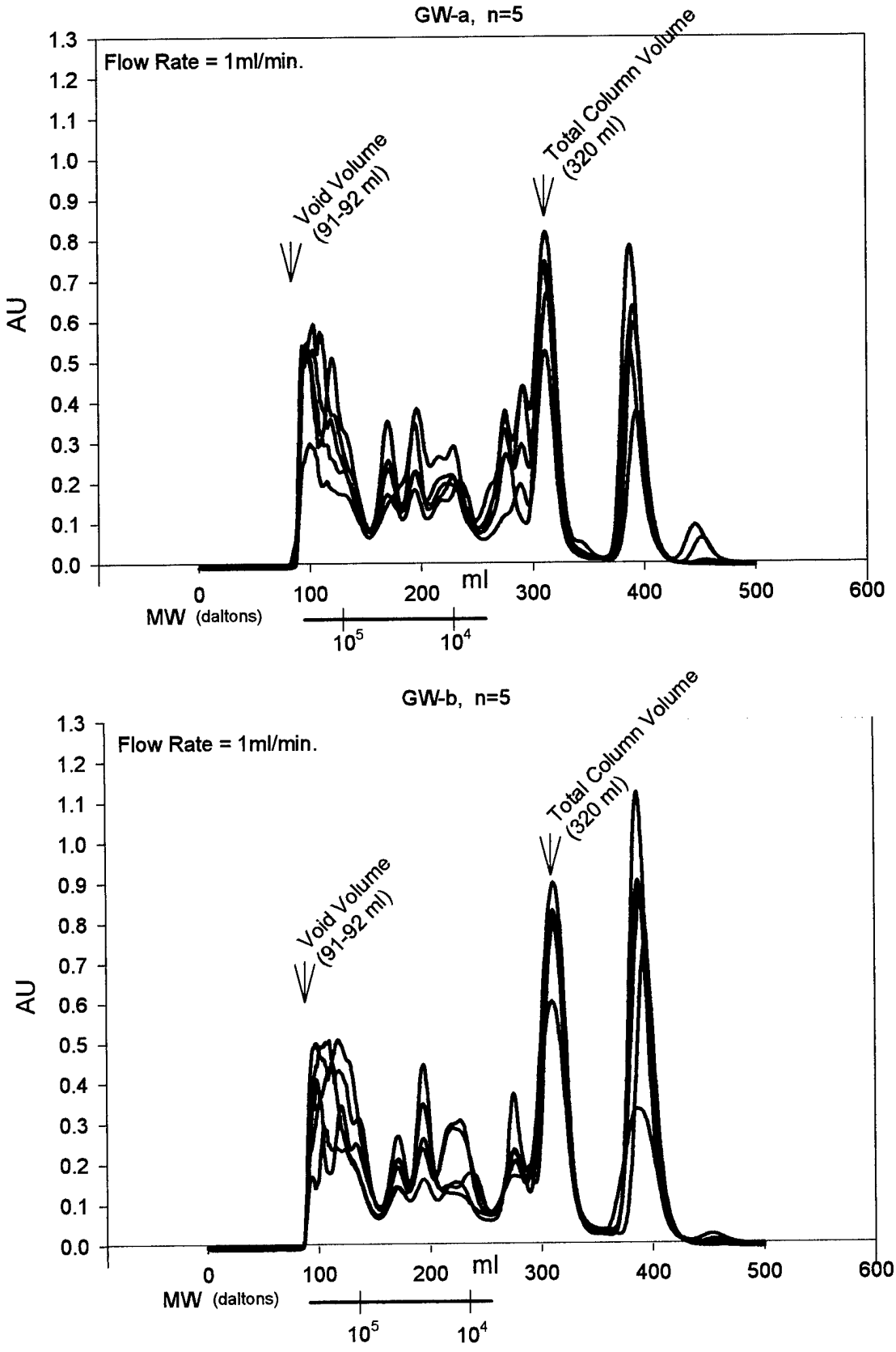


Figure 4. Chromatography of Whole Seminal Plasma from a sample of Civilians with Seminal Plasma Hypersensitivity

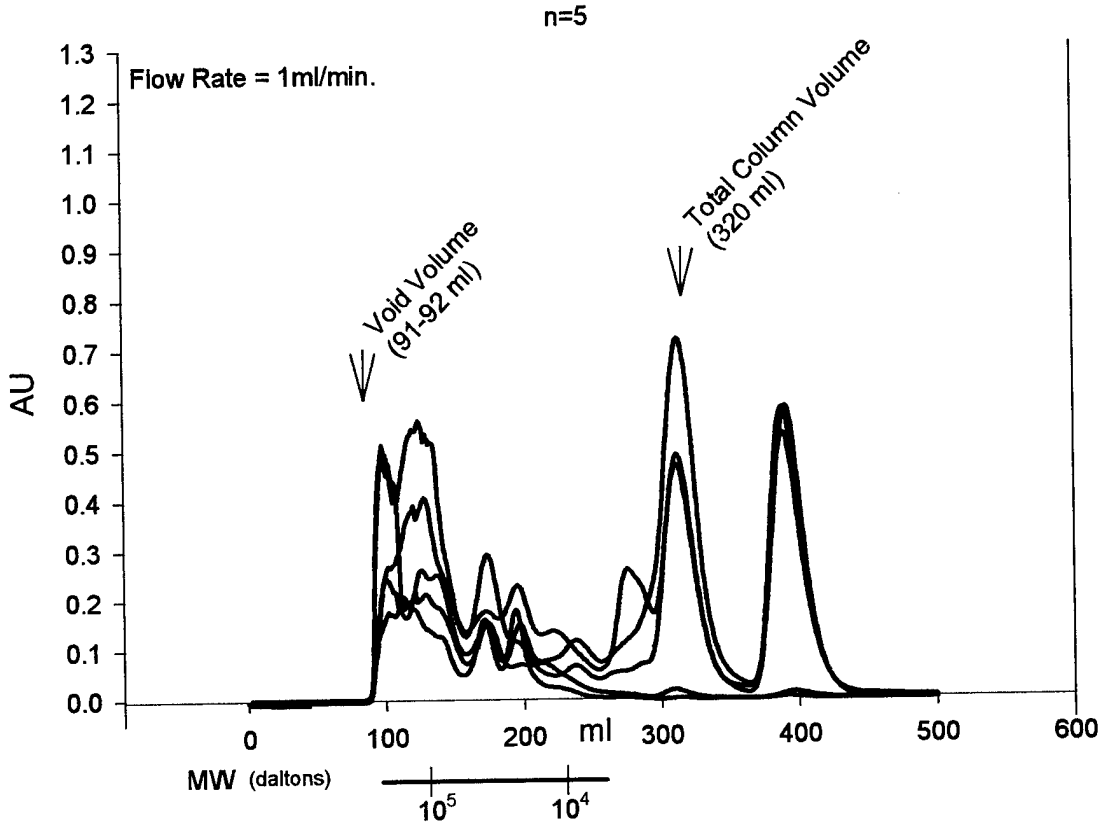


Figure 5. Comparison of IgG and IgE Antibody Responses to Whole SPP in Gulf War& Civilian Couples

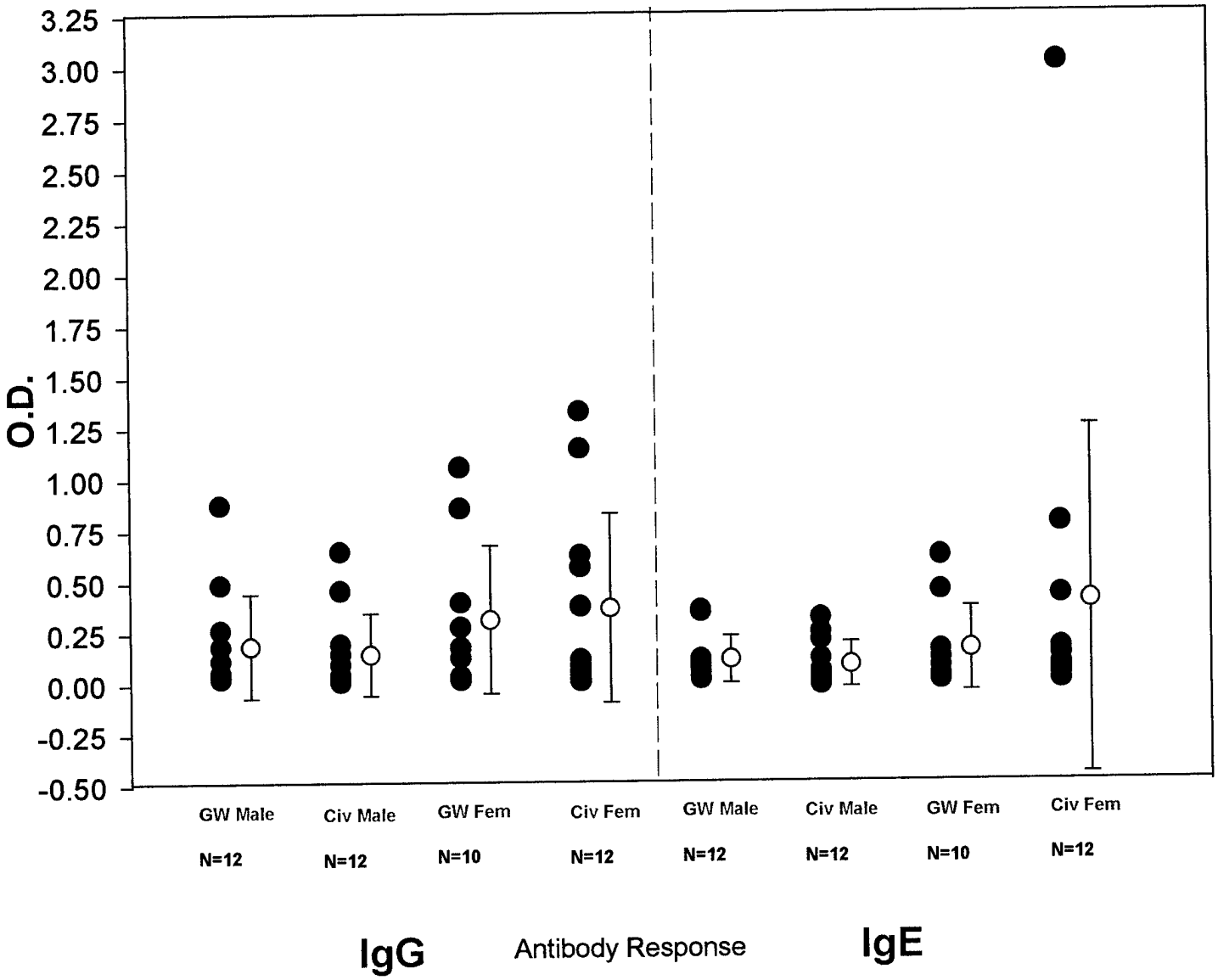
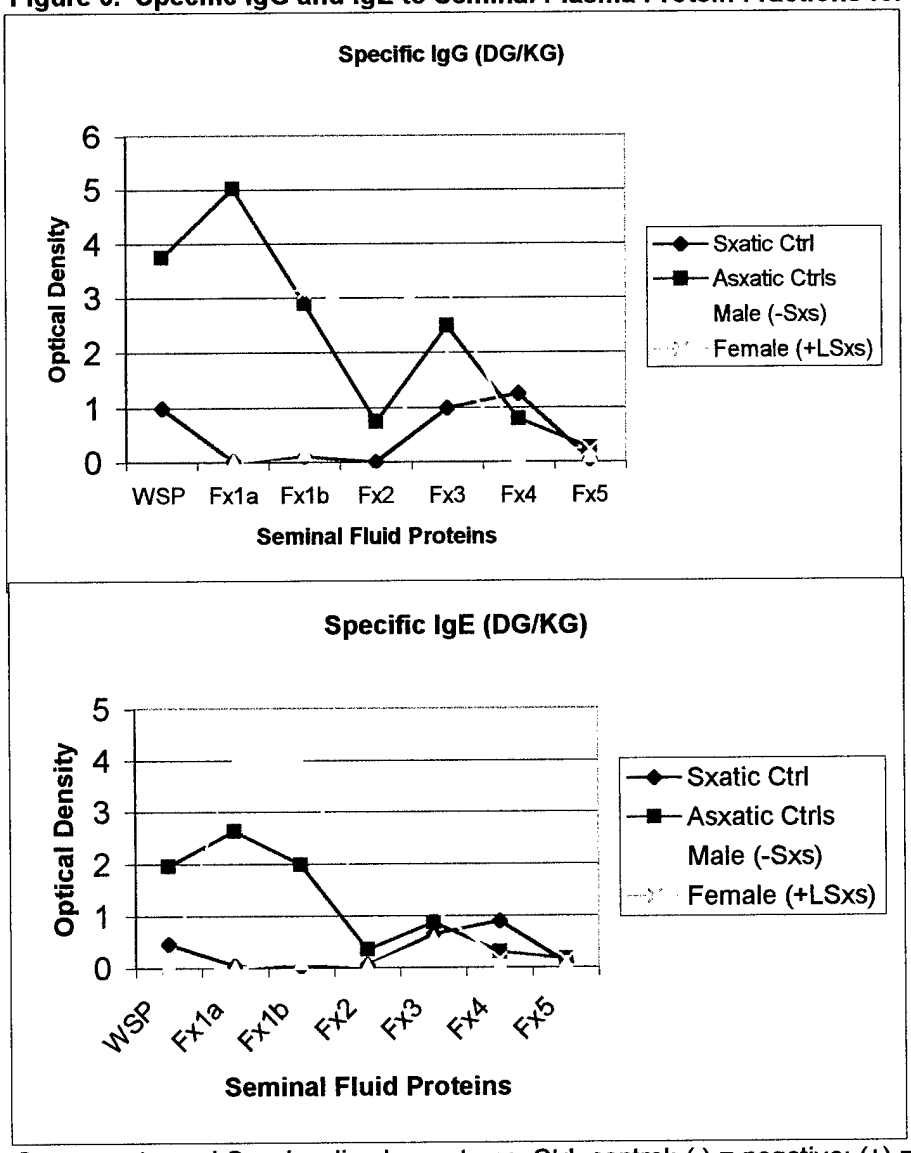
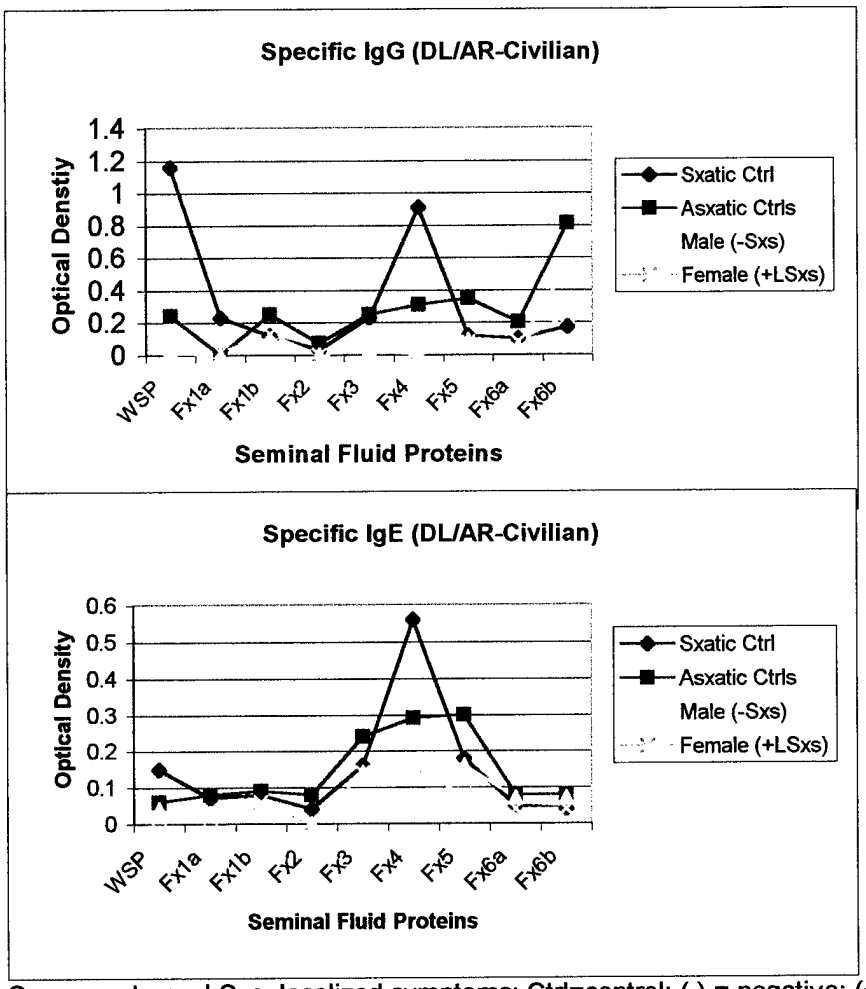


Figure 6. Specific IgG and IgE to Seminal Plasma Protein Fractions for a GW Couple



Sxs=symptoms; LSxs=localized symptoms; Ctrl=control; (-) = negative; (+) = positive

Figure 7. Specific IgG and IgE to Seminal Plasma Protein Fractions for a Civilian Couple



Sxs=symptoms; LSxs=localized symptoms; Ctrl=control; (-) = negative; (+) = positive

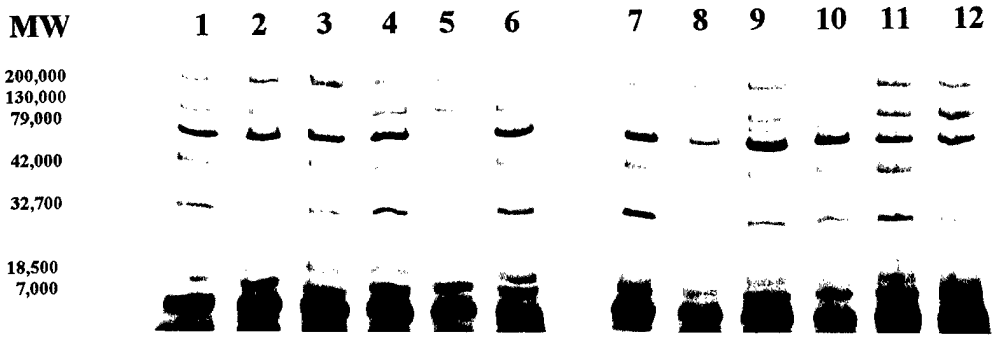


Figure 8. Gel electrophoresis and silver staining of whole seminal plasma from GW veterans and civilian controls. From left to right: Lanes 1-6 and 8-11 are GW specimens; Lanes 7 and 12 are civilian specimens.

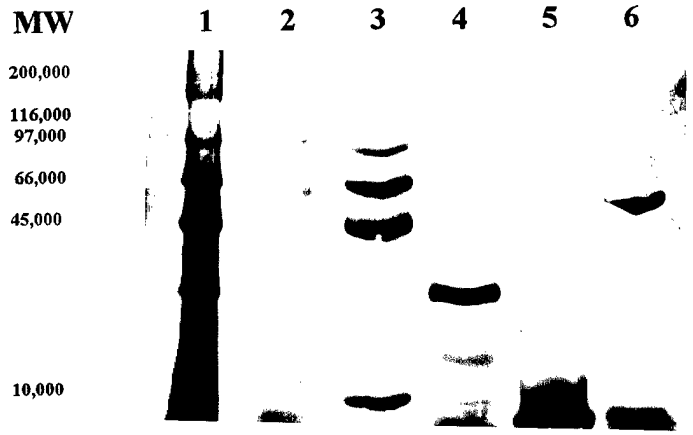


Figure 9. Silver stained SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a GW veteran. Lane 1 is whole seminal plasma, lane 2 is fraction 1a, lane 3 is fraction 1b, lane 4 is fraction 2, lane 5 is fraction 3 and lane 6 is fraction 4.

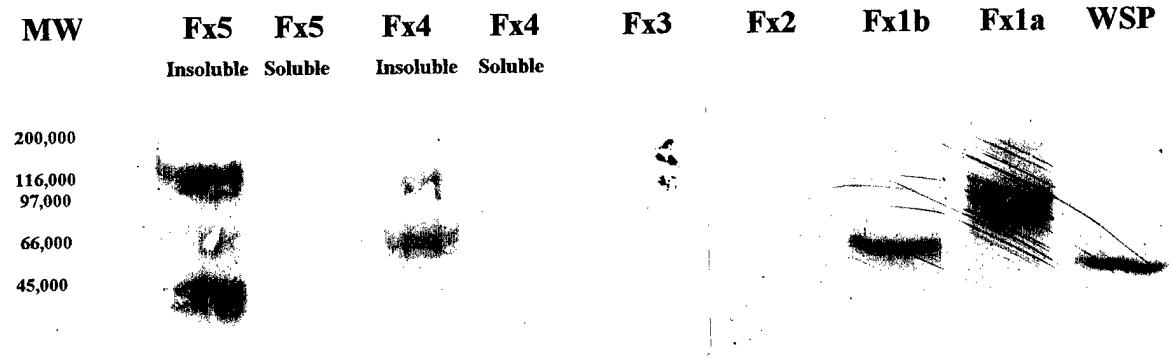


Figure 10. IgG immunoblot of SDS-PAGE gel of GW veteran in figure 4. PVDF membrane incubated with the GW veteran's serum. IgG immunoblotting using the serum of the GW veteran's sexual partner showed similar protein bands for whole seminal plasma and fraction 1b but the other protein bands observed for the GW veteran were not observed (blot not shown).

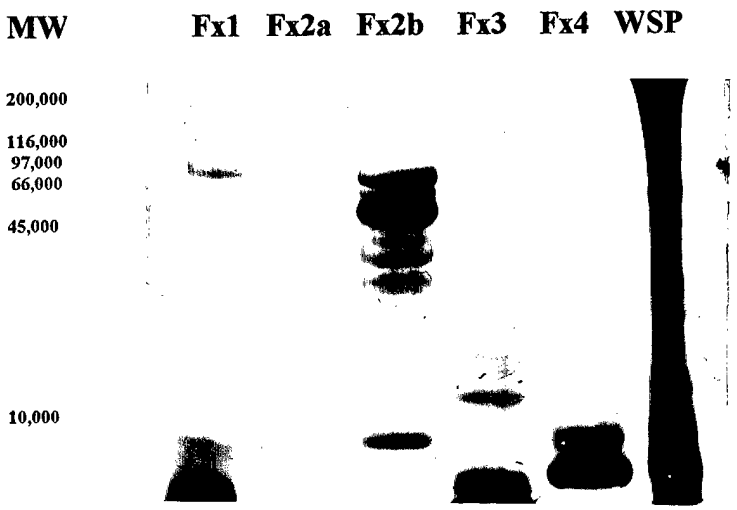


Figure 11. SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a civilian male whose sexual partner was diagnosed with systemic seminal plasma hypersensitivity.

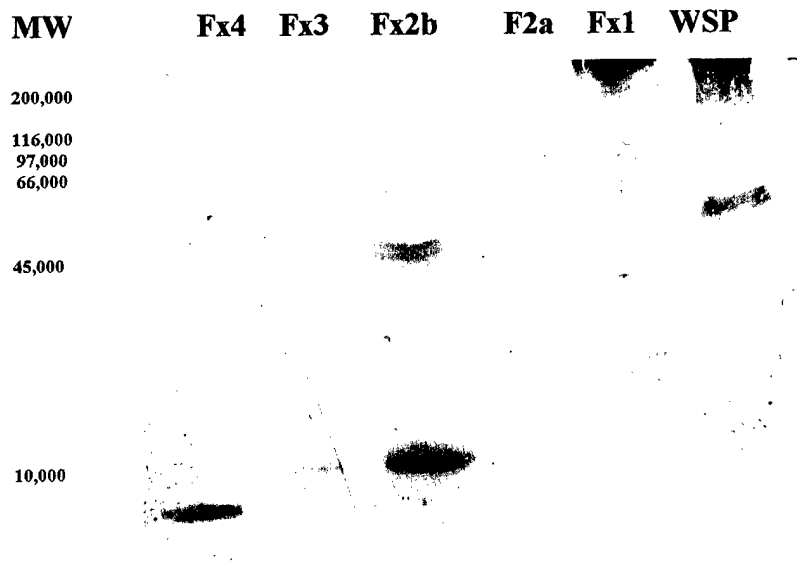


Figure 12 . IgG immunoblot to SDS-PAGE gel in figure 6 using serum of civilian female diagnosed with systemic seminal plasma hypersensitivity and successfully desensitized to fractions 3 and 4.

IX. Appendices

A. Abstracts

1022 LOCALIZED HUMAN SEMINAL PLASMA HYPERSENSITIVITY: A POTENTIAL MODEL FOR GULF WAR "BURNING SEMEN SYNDROME".

J A Bernstein, R L M Martin, Z L Lummus. *University of Cincinnati College of Medicine, Cincinnati, OH.* Sponsor: R E Biagini.

Recently, it has been recognized that the female sexual partners of male Gulf War veterans have experienced localized vaginal symptoms of burning, pain, erythema and swelling after sexual intercourse. These symptoms are completely prevented by barrier contraception. This problem closely resembles localized human seminal plasma (HSP) hypersensitivity which has been well documented in the civilian population. Investigation of civilian women with localized HSP hypersensitivity using indirect ELISA revealed significant levels of specific IgE (OD = 0.29 ± 0.06), IgG (OD = 0.30 ± 0.09), IgA (OD = 0.28 ± 0.07) and IgM (OD = 0.31 ± 0.04) antibodies compared to normal female controls (OD's for IgE, IgG, IgA, IgM:0.06 ± 0.01, 0.01 ± 0.0, 0.05 ± 0.01, 0.13 ± 0.01, respectively) in response to seminal plasma proteins ranging in molecular weight from 41 kD to 220 kD. Furthermore, these women manifest positive skin test reactions to their sexual partner's HSP protein fractions and often symptomatically improve after desensitization using those HSP protein fractions to which they have been sensitized. However, these women have not uniformly responded to desensitization suggesting that other underlying immunologic and/or non-immunologic mechanisms may be involved in addition to IgE-mediated immune responses. In fact, autoantibody to HSP protein has recently been demonstrated in the spouse of a female with localized HSP hypersensitivity. Risk factors for women developing localized HSP hypersensitivity have not been fully elucidated; however, this problem frequently occurs after their first exposure to seminal plasma. Investigation is currently underway to determine whether Gulf War exposure cofactors, such as chemical toxins or infectious agents, are responsible for modifying seminal plasma proteins which can then induce localized vaginal symptoms in the sexual partners of Gulf War veterans in a similar fashion observed in women with localized HSP hypersensitivity.

Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model For Gulf War "Burning Semen Syndrome". Fundamental and Applied Toxicology 1997;37:201.

336 Evaluation Of Persian Gulf War Veterans And Their Sexual Partners With Burning Semen Syndrome. *JA Bernstein,* University of Cincinnati College of Medicine, Cincinnati, OH.

Since returning from the Persian Gulf War (PGW) veterans and/or their wives have reported burning after contact with their semen. This has been called Burning Semen Syndrome (BSS). These reactions bear striking resemblance to reactions experienced by women with localized vaginal seminal plasma hypersensitivity. This project is attempting: 1) to identify PGW couples experiencing BSS; 2) to determine whether these symptoms represent an immunologic, infectious and/or toxicologic etiology; and 3) to determine if there is a causal relationship between BSS and PGW exposures. Screening questionnaires, designed to elicit demographic information, nature of symptoms, Gulf War exposure history and information on post-traumatic stress disorder (PTSD), were distributed to PGW veterans with BSS symptoms. PGW veterans were primarily identified by local and regional Gulf War screening physicians and through a BSS web page on the Internet. There were 46 male respondents. 41 of 46 respondents had sexual partners with vaginal burning after semen contact whereas 15 males experienced burning after contact with their own semen. There was no correlation between BSS and PTSD. Five PGW veterans and their sexual partners had a more extensive evaluation including CBC, differential, chemistries, liver function tests, ANA, sedimentation rate, vaginal/cervical or seminal plasma cultures, skin testing to seasonal and perennial aeroallergens and whole seminal plasma, and specific IgG, IgA and IgE antibodies to seminal plasma proteins by ELISA. Four males and two females were atopic. None elicited a positive skin test or specific antibodies to seminal plasma proteins. Three women grew ureaplasma urealyticum from their cervical cultures, one grew streptococcus Group B, and one Candida. Two women had positive ANA titers (≥ 1:80 titer) and one had an increased sedimentation rate of 65 sec. Larger numbers of PGW veterans and their sexual partners with BSS are currently being evaluated to differentiate between immunologic and infectious etiologies.

Journal of Allergy & Clinical Immunology, 1998; 101:S80.

871 **Antibody Responses in Civilian Couples with Seminal Plasma Protein Hypersensitivity and Gulf War Couples with Burning Semen Syndrome.** *JA Bernstein, AS Perez, KM Frazier, R Floyd,* University of Cincinnati, Cincinnati, OH

Patients with seminal plasma hypersensitivity (SPH) elicit IgG and/or IgE antibody (Ab) to 1 or more seminal plasma proteins (SPP). Gulf War (GW) couples have symptoms (Sxs) similar to SPH called Burning Semen Syndrome (BSS). An ELISA was used to analyze Ab responses for 7 GW couples (GWC) with BSS and 4 civilian couples (CC) with SPH. The average age of GW males (GWM) and GW females (GWF) was 32 yrs and for civilian males (CM) and civilian females (CF) was 35 and 31 yrs, respectively. All CC and 6 GWC were Caucasian and 1 GWC was African American. All CM were asymptomatic. All 7 GWM had localized burning after semen contact but 5 had multiple somatic Sxs characteristic of GW syndrome (GWS). Three CF had localized vaginal burning after semen contact and 1 had systemic Sxs only. Six GWF had localized vaginal burning after semen contact and 1 had multiple Sxs consistent with GWS. Positive Abs were defined as an OD > the mean + 3 SD of 7 negative controls. IgG and IgE Abs to SPP were present in 2 of 4 CF and absent in all CM. The 2 CF with positive Ab responses were successfully desensitized to their sexual partner's SPP. IgG Abs to SPP were found in the male and female of 3 GWC, in only the male of 1 GWC and were absent in 3 GWC. Among the 3 GWC where both partners had IgG Ab, IgE Ab was present in the female of 1 GWC, in the male of 1 GWC and both the female and male of 1 GWC. IgE Ab was absent in the 4 other GWC. For CC, positive IgG and IgE Abs were predictive for successful desensitization but not clinical Sxs. For GWC, there was no correlation between Ab responses and clinical Sxs. These results indicate that GWC with BSS elicit more heterogeneous Ab responses to SPP than CC with SPH. Whether Ab responses in the presence of BSS Sxs predicts successful desensitization to SPP requires further clinical assessment.

Journal of Allergy & Clinical Immunology, 1999;103:S226.

EVALUATION OF PERSIAN GULF WAR VETERANS AND THEIR SEXUAL PARTNERS WITH BURNING SEMEN SYNDROME

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Since returning from the Persian Gulf War (PGW) veterans and/or their wives have reported burning after contact with their semen. This has been called Burning Semen Syndrome (BSS). These reactions bear striking resemblance to reactions experienced by women with localized vaginal seminal plasma hypersensitivity. This project is attempting: 1) to identify PGW couples experiencing BSS; 2) to determine whether these symptoms represent an immunologic, infectious and/or toxicologic etiology; and 3) to determine if there is a causal relationship between BSS and PGW exposures. Screening questionnaires, designed to elicit demographic information, nature of symptoms, Gulf War exposure history and information on post-traumatic stress disorder (PTSD), were distributed to PGW veterans with BSS symptoms. PGW veterans were primarily identified by local and regional Gulf War screening physicians and through a BSS web page on the Internet. There were 96 male respondents. 82 of these respondents had sexual partners with vaginal burning after semen contact and 34 males experienced burning after contact with their own semen. There was no correlation between BSS and PTSD. Five PGW veterans and their sexual partners had a more extensive evaluation including CBC, differential, chemistries, liver function tests, ANA, sedimentation rate, vaginal/cervical or seminal plasma cultures, skin testing to seasonal and perennial aeroallergens and whole seminal plasma, and specific IgG, IgA and IgE antibodies to seminal plasma proteins by ELISA. Four males and two females were atopic. None elicited a positive skin test or specific antibodies to seminal plasma proteins. Three women grew *Ureaplasma urealyticum* from their cervical cultures, one grew streptococcus Group B, and one Candida. Two women had positive ANA titers (1:80 titer) and one had an increased sedimentation rate of 65 sec. Larger numbers of PGW veterans and their sexual partners with BSS are currently being evaluated to differentiate between immunologic, toxicologic, and infectious etiologies.

Keywords: **Burning Semen Syndrome, Seminal Plasma Hypersensitivity**

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SPECIFIC ANTIBODY RESPONSES IN CIVILIAN COUPLES WITH SEMINAL PLASMA PROTEIN HYPERSENSITIVITY AND GULF WAR COUPLES WITH BURNING SEMEN SYNDROME

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Introduction & Hypothesis: Patients with seminal plasma hypersensitivity (SPH) elicit IgG and/or IgE antibody (Ab) to one or more seminal plasma proteins (SPP). Gulf War (GW) couples have symptoms (Sxs) similar to SPH called Burning Semen Syndrome (BSS). It is unclear what the role of specific antibody responses have in the underlying immunopathogenesis of GW couples with BSS.

Methods: An ELISA was used to analyze Ab responses for 10 GW couples (GWC) with BSS and 12 civilian couples (CC) with SPH. Two GW male veterans with BSS without sexual partners were also included.

Demography: The average age of GW males (GWM) and GW females (GWF) was 35 and 34 years, respectively. The average age for civilian males (CM) and civilian females (CF) was 40 and 35 years, respectively. All CC and nine GWC were Caucasian and one GWC was African American. All CM were asymptomatic. Eight GWM had localized burning after semen contact but four had multiple somatic Sxs characteristic of GW syndrome (GWS). Seven CF had localized vaginal burning after semen contact, four CF had vaginal burning and systemic Sxs, and one had systemic Sxs only. Six GWF had localized vaginal burning after semen contact and four had multiple Sxs consistent with GWS.

Results: Positive Ab responses were defined as an OD > the mean + 3 SD of seven negative controls: IgG and IgE Abs to SPP were present in five of 12 CF and in 4 of 12 CM. One CF had IgG Abs only. Only two CF with positive Ab responses underwent treatment. However, both responded successfully to desensitization using their sexual partner's SPP. IgG Abs to SPP were found in the male and female of four GWC, in only the male or female in four GWC and were absent in two GWC and the two GWM without sexual partners. IgE Abs were present in two GWF whose partners were IgG and IgE negative. IgE Ab was absent in the four other GWC and the two GWM without partners. Thus far, only one GWC where both partners had IgG and IgE Abs to SPP underwent desensitization to the male partner's SPP, which was unsuccessful. The female subsequently was successfully treated with Diflucan for a vaginal yeast infection.

Conclusions: For CC, positive IgG and IgE Abs were predictive for successful desensitization. For the one GWC treated thus far, there was no correlation between specific Ab responses and clinical Sxs. Our results indicate that GWC with BSS elicit more heterogeneous Ab responses to SPP than CC with SPH. Larger numbers of GWF with BSS Sxs and specific SPP Ab responses require desensitization to their sexual partner's SPP in order to determine whether humoral immune responses are involved in the pathogenesis of BSS.

KEYWORDS: **Burning Semen Syndrome, Seminal Plasma Hypersensitivity, Antibody Reactions**

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